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REVIEW ARTICLE

The Developing Nervous System: A Series of Review Articles

The following is the sixth in our series of review articles on the developmental biology of the nervous system and its relation to diseases and disorders that are found in newborn infants and children. In this article Dr. Volpe discusses the pathogenesis of periventricular leukomalacia, the major form of brain injury in premature infants. He describes factors that predispose these infants to this disorder as well as potential methods of prevention.

Alvin Zipurksy
Editor-in-Chief

Neurobiology of Periventricular Leukomalacia in the Premature Infant

JOSEPH J. VOLPE

Department of Neurology, Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115, U.S.A.

ABSTRACT

Brain injury in the premature infant is a problem of enormous importance. Periventricular leukomalacia (PVL) is the major neuropathologic form of this brain injury and underlies most of the neurologic morbidity encountered in survivors of premature birth. Prevention of PVL now seems ultimately achievable because of recent neurobiologic insights into pathogenesis. The pathogenesis of this lesion relates to three major interacting factors. The first two of these, an incomplete state of development of the vascular supply to the cerebral white matter, and a maturation-dependent impairment in regulation of cerebral blood flow underlie a propensity for ischemic injury to cerebral white matter. The third major pathogenetic factor is the maturation-dependent vulnerability of the oligodendroglial (OL) precursor cell that represents the major cellular target in PVL. Recent neurobiologic studies show that these cells are exquisitely vulnerable to attack by free radicals, known to be generated in abundance with ischemia–reperfusion. This vulnerability of OLs is maturation-dependent, with the OL precursor cell highly vulnerable and the mature OL resistant, and appears to relate to a developmental window characterized by a combination of deficient antioxidant defenses and active acquisition of iron during OL differentiation. The result is generation of deadly reactive oxygen species and apoptotic OL death. Important contributory factors in pathogenesis interact with this central theme of vulnerability to free radical attack. Thus, the increased likelihood of PVL in the presence of intraventricular hemorrhage could relate to increases in local iron concentrations derived from the hemorrhage. The important contributory role of maternal/fetal infection or inflammation and cytokines in the pathogenesis of PVL could be related to effects on the cerebral vasculature and cerebral hemodynamics, to generation of reactive oxygen species, or to direct toxic effects on vulnerable OL precursors. A key role for elevations in extracellular glutamate, caused by ischemia–reperfusion, is suggested by demonstrations that glutamate causes toxicity to OL precursors by both nonreceptor- and receptor-mediated mechanisms. The former involves an exacerbation of the impairment in antioxidant defenses, and the latter, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor–mediated cell death. Most importantly, these new insights into the pathogenesis of PVL suggest potential preventive interventions. These include avoidance of cerebral ischemia by detection of infants with impaired cerebrovascular autoregulation, *e.g.* through the use of *in vivo* near-infrared spectroscopy, the use of free radical scavengers to prevent toxicity by reactive oxygen species, the administration of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor antagonists to prevent glutamate-mediated injury, or the use of maternal antibiotics or anticytokine agents to prevent toxicity from maternal/fetal infection or inflammation and cytokines. **(*Pediatr Res* 50: 553–562, 2001)**

Abbreviations

PVL, periventricular leukomalacia
WM, white matter
OL, oligodendroglia
CBF, cerebral blood flow
CSF, cerebrospinal fluid
AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
TNF-α, tumor necrosis factor-α

Among all problems in neonatal medicine, brain injury in the premature infant, especially prevention of that injury, is of particular importance. The absolute magnitude of this problem is enormous. Thus, in the United States alone, approximately 57,000 infants are born yearly with a birth weight $<$1500 g (1). Because of major advances in neonatal intensive care, nearly 90% of such infants now survive the neonatal period. The downside is that approximately 10% later exhibit the spastic motor deficits categorized as cerebral palsy, and, importantly, an additional 25– 50% later manifest cognitive or behavioral

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Correspondence and reprint requests: Joseph J. Volpe, M.D., Department of Neurology, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, U.S.A.; e-mail: joseph.volpe@tch.harvard.edu
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deficits that result in serious school disturbances (2–5). This enormous amount of neurologic morbidity relates primarily to the brain injury that is the topic of this review.

Brain injury in the premature infant includes a variety of neuropathologic lesions, including PVL, germinal matrix-intraventricular hemorrhage, posthemorrhagic hydrocephalus, and several patterns of neuronal injury (5). The first two of these lesions are the most important, and with the recently declining incidence in intraventricular hemorrhage, PVL has emerged as the principal form of brain injury in the premature infant (5). Thus, this review will focus on the neurobiology of PVL, with a particular emphasis on the pathogenesis of the lesion and the preventive interventions that are suggested by recent insights into pathogenesis.

NEUROPATHOLOGIC AND CLINICAL FEATURES OF PVL

The neuropathology of PVL consists of two principal components, focal and diffuse (Fig. 1) (5). The focal component, located deep in the cerebral WM, is characterized by localized necrosis of all cellular elements with subsequent cyst formation. The diffuse component is a less severe injury, apparently cell-specific, categorized by diffuse injury to OL precursors. The latter cells, of course, are destined to develop later into mature OLs, which form the myelin of the cerebral WM. The latter process is principally a postterm event in human brain. Thus, not unexpectedly, the principal neuropathologic sequela of PVL is diminution of WM volume and ventriculomegaly, secondary to the deficiency of myelin.

The clinical features of PVL include diagnostic aspects and clinicopathologic correlations (5). Diagnosis of the focal component of PVL is made readily in the neonatal period by cranial ultrasonography. However, the diffuse component of the lesion is invisible to cranial ultrasonography in the neonatal period. Diffusion-weighted magnetic resonance imaging has been shown to identify this lesion (6), although more data are needed on sensitivity and specificity of diffusion-weighted imaging. Diagnosis of the later deficit of myelin and the ventriculomegaly is made readily by conventional brain imaging. Available qualitative imaging data suggest that the diffuse component of PVL is considerably more common than is the focal component, although quantitative information is lacking (5). The principal clinicopathologic correlates of PVL are spastic diplegia, related primarily to the deep periventricular locus of the focal component of the lesion, and the cognitive and behavioral deficits, related, I believe, to the more diffuse component of the lesion.

PATHOGENESIS AND NEUROBIOLOGY OF PVL

The pathogenesis of PVL consists of at least three major interacting factors. The first two of these factors underlie a propensity for the occurrence of cerebral ischemia, and the third of these factors concerns the particular vulnerability of OL precursors to ischemia and, importantly, to other related insults, as I will discuss.

Vascular Anatomic and Physiologic Factors

The focal and diffuse components of PVL appear to relate in part to the development of the vascular supply to the cerebral WM (7–11). This supply consists principally of the long and short penetrating arteries (Fig. 1). Thus, the focal component of PVL with loss of all cellular elements occurs principally in the distribution of the end zones of the long penetrating arteries (Fig. 1). The distal fields of these vessels are not fully developed in the premature infant, and, thus, with decreases in CBF these areas would be subjected to severe ischemia. The diffuse

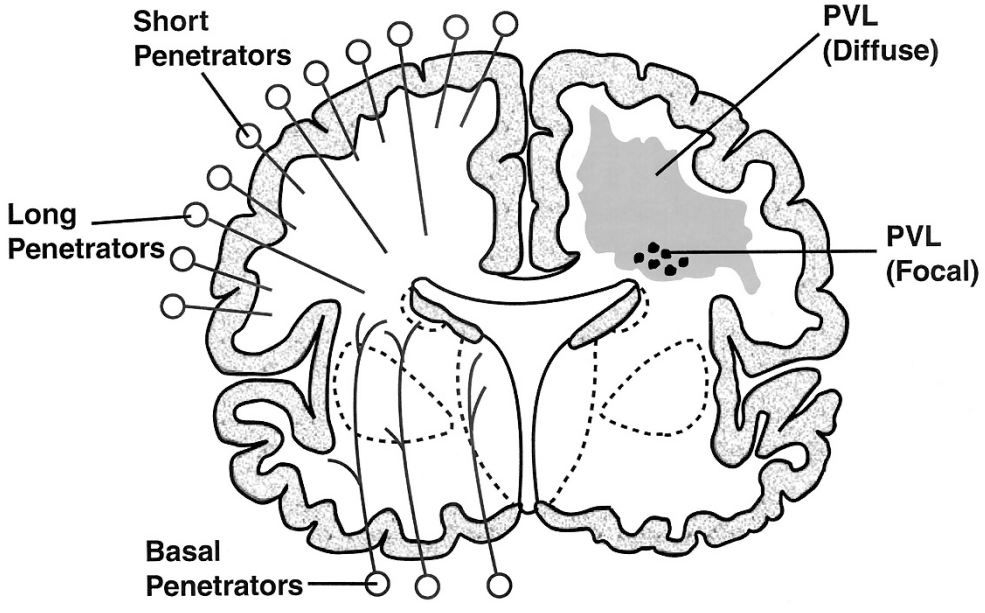

Figure 1. Schematic depiction of coronal section of cerebrum with the focal and diffuse components of PVL shown in one hemisphere and the cerebral vascular supply in the other hemisphere. The focal necrotic component of PVL is depicted by the black circles, and the diffuse OL-specific component, in the gray shading. The long and short penetrating arteries supply the cerebral WM, as shown.

OL-specific component of PVL occurs principally in the distributions of *1*) the border zones between the individual long penetrating arteries and *2*) the end zones of the short penetrating arteries. The short penetrating arteries do not develop fully until the cerebral cortex develops fully in the postterm period. Thus, with declines in CBF, moderate ischemia and the more cell-specific loss of OL precursors would be expected.

A physiologic correlate of these vascular anatomic factors appears to be the extremely low blood flow to cerebral WM in the human premature newborn, first shown clearly by work with positron emission tomography (12). The finding of extremely low WM flows is consistent with measurements of mean global CBF in ventilated human premature infants (13–19). The studies of regional CBF by positron emission tomography showed that values in cerebral WM in surviving preterm infants with normal or near normal neurologic outcome ranged from only 1.6 to 3.0 mL·100 g^{-1}·min^{-1} (12). These remarkably low values in WM were approximately 25% of those in cortical gray matter, a regional difference later confirmed in a study using single photon emission tomography (20). The blood flow values of $<$5.0 mL·100 g^{-1}·min^{-1} in normal or near normal cerebral WM in the preterm infant are markedly less than the threshold value for viability in adult human brain of 10 mL·100 g^{-1}·min^{-1} (normal CBF in the adult is approximately 50 mL·100 g^{-1}·min^{-1}) (21). The very low values of volemic flow in cerebral WM in the human premature infant suggest that there is a minimal margin of safety for blood flow to cerebral WM in such infants.

Thus, these maturation-dependent cerebrovascular factors, coupled with the neuropathology of PVL discussed earlier, suggest that the focal necroses, affecting all cellular elements and localized to deep cerebral WM, are related to relatively severe ischemia. The more peripheral diffuse cerebral WM injury, affecting, apparently specifically, OL precursor cells, although relatively sparing other cellular elements, may be related to less severe ischemia.

Impaired Cerebrovascular Autoregulation or Pressure-Passive Cerebral Circulation

The vascular end zones and border zones just described thus would render the premature infant's brain particularly vulnerable to injury in the presence of cerebral ischemia. Perhaps of particular importance in the genesis of impaired CBF and thereby cerebral ischemia is an apparent impairment of cerebrovascular regulation in at least a subset of ventilated premature infants. This impairment was suggested initially by studies using the invasive technique of radioactive xenon clearance (22–25). Thus, in such sick premature infants with a pressure-passive cerebral circulation it would be expected that when blood pressure falls, as occurs commonly in such infants, so would CBF, with the consequence being ischemia in the distribution of the arterial end zones and border zones in cerebral WM. Moreover, the particular danger is compounded by the demonstration that blood flow to cerebral WM of the infant is very low (see earlier) and that thereby a minimal margin of safety may exist.

Clinically stable premature infants seem less likely to exhibit this apparent lack of cerebrovascular autoregulation (13, 14, 24, 26, 27), although some studies of such "healthy" premature infants identify "absent" autoregulation even in this setting (28). With intact cerebrovascular autoregulation in the mature child or adult, CBF remains constant over a wide range of blood pressure because of arteriolar dilation with decreases in blood pressure and arteriolar constriction with increases in blood pressure. Studies in preterm lambs suggest that during the maturation of cerebrovascular autoregulation there is an early phase in which the range of blood pressure over which CBF is maintained constant, although present, is narrow and that the normal blood pressure is near the downslope of the autoregulatory curve (29, 30). Such a situation would render even the premature infant with a degree of intact autoregulation vulnerable to modest declines in blood pressure. The significance of such modest declines could go undetected if the focus of monitoring were systemic blood pressure alone and not the relation between blood pressure and the cerebral circulation.

A relation between impaired CBF and the occurrence of PVL is supported further by clinical studies that relate the lesion to neonatal events expected to cause cerebral ischemia. Such events associated with the development of PVL include severe hypotension, marked hypocarbia, hypoplastic left heart syndrome, patent ductus arteriosus with retrograde cerebral diastolic flow, and severe illness requiring extracorporeal membrane oxygenation, among others [see Volpe (5) for review].

The difficulty in conclusively establishing a relationship between a pressure-passive cerebral circulation and the occurrence of PVL is related to the inability to determine which infants exhibit such a hemodynamic abnormality and if so, whether such infants develop PVL. The methodological hurdle has been the inability to measure quantitative changes in the cerebral circulation from second to second. The advent of *in vivo* near-infrared spectroscopy has changed this situation. Thus, this noninvasive technique, now near the threshold of clinical application, allows the measurement, essentially in real-time, of cerebral concentrations of oxygenated and deoxygenated Hb (5). Changes in the concentrations of these two intravascular compounds provide information about cerebral blood volume, CBF, and oxygen delivery (31, 32). Using this technique in a preliminary study of 32 mechanically ventilated premature infants from the first hours of life, we identified a pressure-passive cerebral circulation (Fig. 2) in 53%; such infants had approximately a fourfold increased risk of PVL or severe intraventricular hemorrhage and accounted for the vast majority of all examples of these severe lesions (33). Although the numbers are small and the data preliminary, the observations suggest that premature infants with a pressure-passive cerebral circulation are at high risk for the development of ischemic WM injury and that such infants can be identified before the occurrence of such injury. Future work must be directed at confirmation of these preliminary results, identification of the causes of the cerebral circulatory abnormality, and formulation of a means of preventing this disturbance.

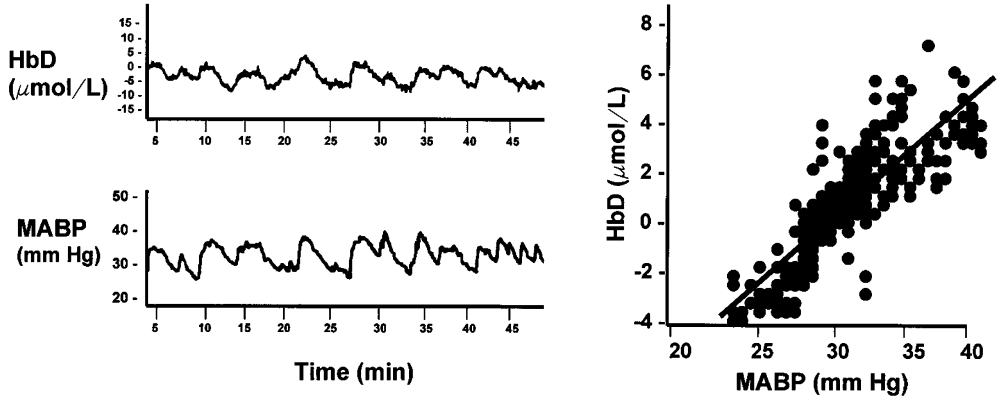

Figure 2. Pressure-passive cerebral circulation in a premature infant of 26-wk gestation, on the second postnatal day. The upper trace was obtained from the cerebral circulation by near-infrared spectroscopy and the lower trace from the umbilical artery transducer for mean arterial blood pressure (MABP). HbD is the difference value of HbO_2 − Hb, shown in animal studies to correlate tightly with cerebral blood flow (31, 32). The right panel shows a plot of the data points and the nearly linear relationship between MABP and HbD. The infant later exhibited the ultrasonographic features of PVL.

Maturation-Dependent Vulnerability of OL Precursors

OL precursor as the key cellular target in PVL. Specific maturation-dependent characteristics of the actively differentiating OL precursors in the human cerebrum appear to be very important in the pathogenesis of particularly the diffuse component of PVL. A vulnerability of immature WM to hypoxia-ischemia has been suggested by studies of hypoxic-ischemic injury in midgestation fetal sheep, late-gestation fetal sheep, near-term fetal sheep, 1-d-old piglets, and 5- and 7-d-old rat pups (34–43). These observations led us to investigate the neurobiologic mechanisms of the intrinsic vulnerability of OL precursors in a highly defined system of cultured OLs. Before undertaking those studies in depth we set out to determine the specific stage in the OL lineage present in the cerebral WM of the human premature infant and the presumed target, therefore, of the diffuse OL injury in PVL. Our studies identified the dominant form of OL in the WM of the human premature infant, and perhaps therefore the key cellular target in PVL, as an early differentiating OL with the specific immunocytochemical characteristics of an OL precursor (44). With this information concerning human brain we developed a chemically defined culture system to study, in nearly pure form, the different stages of the OL lineage, including the OL precursor stages (45). With this system of nearly pure OL cultures, we have addressed four important questions, as follows. First, are OLs particularly vulnerable to free radical attack? Second, what is the mode of cell death caused by such free radical attack? Third, is any vulnerability to free radical attack maturation-dependent? Fourth, what are the mechanisms underlying the maturation-dependence of this vulnerability?

Vulnerability of OL precursors to free radical attack. We asked first the question of the vulnerability of OLs to free radical attack, because PVL has been considered to be an ischemic lesion, and an elevation in a variety of reactive oxygen species is a well-established sequela of ischemia–reperfusion (46–49). Direct and indirect evidence for increases in oxygen free radicals in developing brain during reperfusion after hypoxia-ischemia has been obtained in studies of neonatal and fetal animals (50–66). This evidence includes direct demonstrations of elevated free radicals, as well as amelioration of deleterious neural effects by the use of free radical scavengers or inhibitors of free radical formation. The nature of the specific free radicals involved varies somewhat with the experimental model but principally includes initially superoxide anion and hydrogen peroxide (67). Although derivatives of nitric oxide, such as peroxynitrite, have been implicated in some paradigms, we have shown that nitric oxide is protective to OL precursors under conditions of oxidative stress (68). In two model systems of free radical accumulation, we have shown that OL precursors in culture indeed are exquisitely vulnerable to free radical attack (45, 69, 70). Moreover, clinically safe free radical scavengers, *e.g.* vitamin E, totally prevented the OL death caused by free radical attack. Interestingly, vitamin E was capable of rescuing OL precursors from free radical-mediated death even when added many hours after onset of the insult (unpublished data).

Having demonstrated that early differentiating OLs are exquisitely vulnerable to free radical-mediated cell death, we next set out to determine the mode of cell death, because the specific form of cell death may provide valuable insights into the molecular mechanisms. The determination of specific mode of death is relevant to periventricular WM injury, in part because findings obtained in several neuronal systems suggest that a moderate insult leads to neuronal death by apoptosis and a severe insult, to death by necrosis (71, 72). As discussed earlier the diffuse OL injury in PVL is likely to be related to moderate ischemia, as contrasted with the severe ischemia in deep periventricular WM that results in focal necrosis with loss of all cellular elements. Moreover, studies in the neonatal piglet subjected to hypoxia-ischemia have demonstrated exclusively necrotic cell death in certain neuronal populations, both necrosis and apoptosis in other neuronal populations, but exclusively apoptotic cell death in immature cerebral WM (34). Similarly, the chromatin clumping and nuclear condensation so characteristic of the "acutely damaged glia" of the diffuse component of human PVL (73, 74) also suggest the possibility of apoptotic cell death. Consistent with all of these data, our studies of OL precursors subjected to free radical attack in

culture in fact show features consistent with apoptosis as the mode of cell death, *i.e.* margination of chromatin, nuclear condensation, preservation of plasma membrane, oligonucleosomal DNA fragmentation (by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling), and prevention by protein synthesis inhibitors (45).

We next asked whether the exquisite vulnerability of OLs to free radical attack and cell death is maturation-dependent. Using the stage-specific system of OLs in culture and the cystine deprivation (and thereby glutathione depletion) model of free radical attack, we have shown that the early differentiating OL is vulnerable to free radical attack, whereas the mature OL is resistant (Fig. 3*A*) (45). The difference in vulnerability was apparent in a separate model of exposure to free radicals (45). This maturation-dependent vulnerability thus may be critical for the predilection of this lesion for the human brain early in life and the absence of the lesion in similar form after OL maturation and myelination occur.

The last of the four questions raised earlier, *i.e.* the mechanisms underlying the maturation-dependence of the vulnerability of OL precursors to free radical attack, is perhaps the most important. Direct comparison of OL precursors and mature OLs under conditions of free radical attack showed that the precursor cells accumulate free radicals whereas the mature cells do not (Fig. 3*B*). The OL precursors appear deficient in capability of handling the free radicals. The potential explanation for this deficiency is suggested from information derived from studies of experimental models (61, 65, 66, 75–83) and limited analyses of autopsied human brain (84–86). Taken together the findings suggest a delay in the development and the reactivity of antioxidant defenses, especially glutathione peroxidase and catalase (Fig. 3*C*). The latter enzymes are involved in detoxification of hydrogen peroxide. As shown in Fig. 3*C*, when these defenses fail or are overwhelmed, hydrogen peroxide accumulates, and in the presence of Fe^{2+} the Fenton reaction produces the deadly hydroxyl radical. Early in differentiation OLs are likely to accumulate iron because of the active acquisition of iron required for OL differentiation and probably also because of the accumulation of nonprotein-bound iron as a consequence of hypoxic-ischemic insult. Consistent with these findings, we showed that cultured OL precursors were totally resistant to free radical attack in the presence of the iron chelator, desferrioxamine (45). Supporting the relevance of these findings to the human infant are studies of plasma of human premature infants suggesting both a propensity to generate free radicals, including the hydroxyl radical, and impaired antioxidant defenses (87–96). Moreover, the first reported study of peroxidation products in CSF of premature infants shows elevations in infants with subsequent evidence of WM injury by magnetic resonance imaging, compared with levels in infants without WM injury (97).

Thus, the proposed relationship between ischemia–reperfusion and cell death in OL precursors is provided in Figure 3*D*. A maturation-dependent window of vulnerability

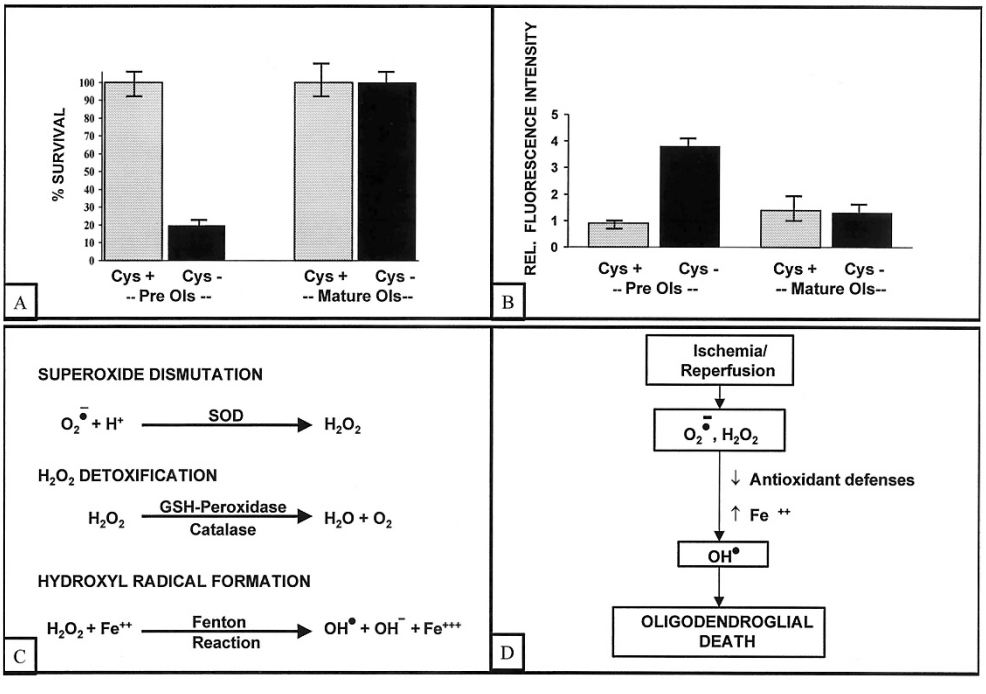

Figure 3. Vulnerability of cultured OL precursors to free radical attack. *A*, free radicals are more toxic to OL precursors (*Pre OLs*) than to mature OLs. Free radical attack is produced by 24 h of growth in cystine-deprived medium (*Cys*−), which results in glutathione depletion. *B*, free radicals accumulate in OL precursors but not in mature OLs with cystine deprivation. Glutathione levels declined to the same nadir in both cell types (data not shown). Free radicals were determined by a fluorescence technique. *C*, free radical metabolism with ischemia–reperfusion. The superoxide anion is generated and undergoes conversion to hydrogen peroxide (H_2O_2) by the action of superoxide dismutase (*SOD*). Hydrogen peroxide is detoxified by catalase and glutathione (*GSH*) peroxidase. If this detoxification step fails or hydrogen peroxide accumulates, and if Fe^{2+} is available, the Fenton reaction can produce the deadly hydroxyl radical ($OH^{\bullet}$). *D*, summary scheme for pathogenesis of OL death under conditions of ischemia–reperfusion. The central role of free radical attack and the basis of the vulnerability of OL precursors (impaired antioxidant defenses and acquisition of Fe^{2+}) are shown.

appears likely. The central roles of developmentally deficient antioxidant defenses, acquisition of iron for differentiation, and free radical accumulation are shown.

Potential Role of Hemorrhage in OL Death

The particular propensity for the occurrence of intraventricular hemorrhage in the premature infant may accentuate this apparent maturation-dependent vulnerability to free radical attack. Thus, the incidence of PVL is higher in infants who sustain intraventricular hemorrhage *versus* those who do not, whether brain is studied postmortem (98, 99) or in the living infant (5, 100, 101). Although several reasons for this relationship seem possible, including similarities of the pathogenesis of the two lesions (5), an excellent possibility is that the hemorrhage provides a rich source of iron for the generation of reactive oxygen species (Fig. 4). Supportive of this suggestion is the recent demonstration that nonprotein-bound iron was found in CSF of 75% of preterm infants with posthemorrhagic ventriculomegaly but was not found in CSF of infants without prior hemorrhage (102).

Potential Role for Glutamate in OL Death

Potential for increased extracellular glutamate. A potential role for excess extracellular glutamate in the pathogenesis of PVL is suggested by several interrelated observations. First, an elevation of extracellular glutamate is likely in cerebral WM subjected to hypoxia-ischemia. The mechanisms for such an elevation are multiple. Thus, the earliest and an especially prominent neuropathologic feature of focal PVL is coagulation necrosis and disruption of axons (103, 104). Additionally, in both human and experimental models of PVL, immunocytochemical studies suggest that axonal injury can occur without overt focal necrosis (105, 106). Because neurons, and presumably axons, contain millimolar concentrations of glutamate (107–109), it appears likely that substantial amounts of glutamate could leak into the extracellular space on disruption. Additional sources of glutamate with brain ischemia–reperfusion include failure of glutamate uptake in astrocytes and neurons, reversal of glutamate transporter function in astrocytes and OLs, and cytokine effects on astrocytes, among other factors (110, 111).

Nonreceptor-mediated glutamate-induced OL death. Potential importance for elevations in extracellular glutamate is suggested by the demonstrations that glutamate can lead to death of OL precursors both by nonreceptor-mediated and receptor-mediated mechanisms (Fig. 4). Concerning the nonreceptor-mediated mechanism, glutamate causes glutathione depletion in OL precursors and thereby free radical-mediated cell death (69). This effect is mediated by activation of a glutamate-cystine exchange transporter, such that glutamate uptake results in cystine efflux, intracellular cystine depletion, and thereby impaired glutathione synthesis (69). The result is free radical-mediated death, which can be totally prevented by such free radical scavengers as vitamin E (69).

Receptor-mediated glutamate-induced OL death. In addition to the nonreceptor-mediated mechanism just described, activation of the AMPA/kainate type of glutamate receptor can

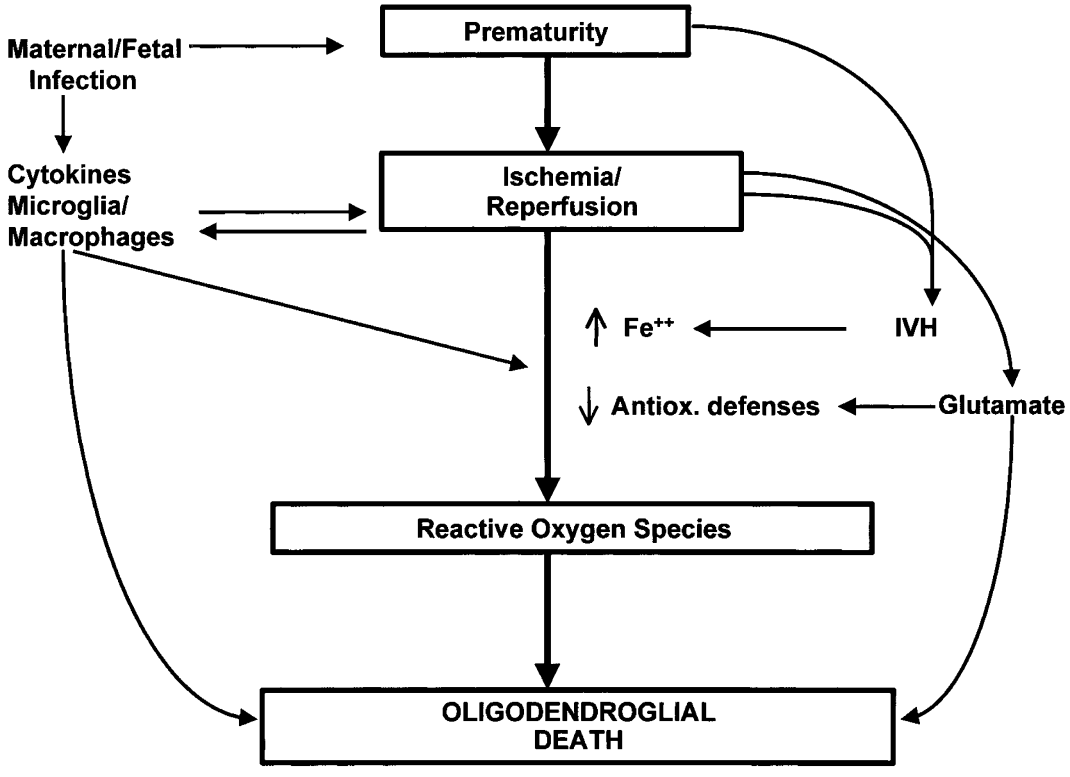

Figure 4. Pathogenesis of OL death in PVL. The scheme shows the central role of vulnerability to free radical attack and the means by which intraventricular hemorrhage (*IVH*), extracellular glutamate, and maternal/fetal infection or inflammation may interact with this central vulnerability.

lead to OL death in culture and *in vivo* (Fig. 4) (41, 112–119). Moreover, our data indicate that this AMPA/kainate form of OL death occurs only in the developing OL and not in the mature OL (115). The relevance of this phenomenon to hypoxic-ischemic cerebral WM OL injury was shown by the demonstration that in the immature rat such injury is prevented by the systemic administration of the non-*N*-methyl-D-aspartate receptor antagonist 6-nitro-7-sulfamoylbenzo(f)quinoxaline-2,3-dione (NBQX) after termination of the insult (41). A similarly beneficial effect in this model has been shown recently with a clinically safe anticonvulsant drug, topiramate, which acts at the AMPA/kainate receptor (120). Thus, taken together these findings indicate that glutamate leads to toxicity of OL precursors by both receptor and nonreceptor-mediated mechanisms. Both mechanisms can be counteracted, the latter by free radical scavengers and the former by specific receptor antagonists.

Maternal/Fetal Infection or Inflammation and Cytokine Release in OL Death

An additional and interrelated mechanism for the death of OL precursors in PVL involves the action of maternal/fetal infection, inflammation, and cytokines (Fig. 4). An important series of clinical, epidemiologic, neuropathologic, and experimental studies suggests that maternal/fetal infection, inflammation, or cytokines are involved in the pathogenesis of a proportion of cases of PVL. Thus, a role for maternal/fetal infection, endotoxin, and presumably endotoxin-mediated cytokine release in the pathogenesis of periventricular WM injury was suggested initially by neuropathologic and epidemiologic studies of human infant brain and by related experimental studies of Gilles and Leviton and coworkers approximately 25–30 y ago (121–123). Two recent demonstrations of cerebral WM lesions in fetal rabbits after the induction of maternal intrauterine infection are consistent with the earlier observations (124, 125). Several recent human studies lend further support to a contributory role for such factors in the pathogenesis of PVL. Thus, the incidence of PVL and cerebral palsy in premature infants is increased in the presence of *1*) evidence for maternal, placental, or fetal infection (126–138), *2*) elevated levels of IL-6 in cord blood (139), *3*) elevated levels of IL-6 and IL-1β in amniotic fluid (140), and *4*) elevated levels of all interferons and IL-1 and IL-6, among other cytokines, in neonatal blood (141–143). Moreover, although potentially a secondary effect of ischemia (see later), the demonstration of IL-6 and TNF-α within PVL lesions is also possibly supportive of a relation of PVL to intrauterine infection and cytokines (144–146).

The possibility of direct injury to developing OLs by cytokines or other bacterial products is raised by studies of cultured OLs (Fig. 4). Thus, some studies, although not all, suggest that TNF-α is toxic to OLs (147–154). Our preliminary data with developing OLs show little or no toxicity to TNF-α in pure OL cultures but high toxicity by interferon-γ. The latter observation has been made by others (150, 155). Moreover, it has been shown that immature OLs in culture are more vulnerable to the cytotoxicity of interferon-γ than are mature OLs (155, 156). Additionally, TNF-α potentiates this toxicity of interferon-γ to developing OLs (153). Finally, a role for cytokines and inflammatory cells in the pathogenesis of cerebral OL injury in the absence of infection also must be considered. Thus, it is well-established in animal models that ischemia–reperfusion is accompanied rapidly by activation of microglia, secretion of cytokines, and mobilization, adhesion, and migration of macrophages and inflammatory cells (Fig. 4). Multiple cytokines, microglia, or white blood cells can be involved. Indeed in one model of excitotoxic injury to developing WM, potentiation of toxicity by several inflammatory cytokines and a central role for microglia have been shown (157, 158). Whether induced by infection or ischemia, these inflammatory responses could be particularly detrimental to developing OLs because production of reactive oxygen species is one mechanism for the cytotoxicity caused by these factors (49, 159–163). Thus, the central theme of vulnerability of OL precursors to such reactive species may be unifyingly relevant in this context (Fig. 4).

Infection and cytokines, individually or in combination, may lead to ischemia–reperfusion and thereby the potential for OL injury on that basis (Fig. 4). Thus, a distinct disturbance of vascular endothelium can be produced by endotoxin, as observed in brains of newborn kittens that developed PVL after endotoxin injection (122, 164, 165). Additionally, endotoxin has been shown to cause arterial hypotension in newborn dogs, in sublethal doses, and to produce in the same animals periventricular WM injury (166). Moreover, in the model the deficits in blood flow and metabolism produced in cerebral WM by hemorrhage-induced hypotension were similar to those produced by endotoxin-induced hypotension. Related work in immature rabbits also showed a particular propensity for cerebral WM to develop both decreased CBF 1 to 2 h after endotoxin administration and histologic evidence for necrosis subsequently (167). Finally, because of the pronounced vasoactive effects of certain cytokines (*e.g.* TNF-α) and of other compounds (*e.g.* nitric oxide) released as part of the inflammatory cascade (168), an impairment of cerebrovascular regulation and thereby risk for ischemic injury also could become operative (Fig. 4). Further data in developing animals would be of particular interest.

FROM PATHOGENESIS TO PREVENTION

Consideration of the pathogenetic scheme depicted in Figure 4 raises the possibility of several promising interventions to prevent PVL. Especially critical is maintenance of cerebral perfusion. Detection of the infant with impaired cerebrovascular autoregulation by the use of near-infrared spectroscopy is likely to be valuable. Avoidance of factors that may lead to cerebral ischemia even in the presence of intact autoregulation, *e.g.* severe hypotension or marked hypocarbia, or that may impair intact autoregulation, *e.g.* moderate hypoxemia or marked hypercarbia, is important.

Perhaps of greatest value is prevention of the cascade to OL death related to free radical attack (Fig. 4). Thus, the use of clinically safe free radical scavengers, *e.g.* vitamin E, could be beneficial, after further research. Maternal antimicrobials and anticytokine agents may ultimately prove valuable in prevent-

ing the injury caused by maternal/fetal infection or inflammation and cytokines (Fig. 4). Antagonists of the AMPA/kainate glutamate receptor might be effective if a clinically safe agent, *e.g.* topiramate, can be identified (Fig. 4). Antiapoptotic agents, such as neurotrophins, growth factors, or specific inhibitors of proapoptotic pathways, will require further delineation of the specific final molecular pathways to OL death in this setting. Agents that may act at multiple sites in the pathogenetic scheme shown in Figure 4, are administered antenatally, and have been suggested to be beneficial in prevention of PVL include magnesium sulfate and glucocorticoids. Magnesium sulfate has vasodilator, antioxidant, and anticytokine effects, but its potential benefit and safety are controversial (5, 169). Similarly, enthusiasm for antenatal glucocorticoids (170, 171) must be tempered by the recent demonstrations of deleterious cerebral effects of antenatal dexamethasone (but not betamethasone) (172) and of postnatal dexamethasone (173–175).

It is likely that ultimately combinations of interventions will prove most effective in prevention of the WM injury of PVL. With the recent insights into pathogenesis of PVL and the neurobiology of OL precursors in this pathogenetic context (Fig. 4), the critical sites for interventions are becoming clarified. Fruitful clinical trials can now be seen on the horizon.

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